

This Page Is Inserted by IFW Operations  
and is not a part of the Official Record

## **BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning documents *will not* correct images,  
please do not report the images to the  
Image Problem Mailbox.**

**WEST**[Help](#)[Logout](#)[Interrupt](#)[Main Menu](#)[Search Form](#)[Posting Counts](#)[Show S Numbers](#)[Edit S Numbers](#)[Preferences](#)**Search Results -**

Terms	Documents
wo89/12694.ptpn.	0

☐ US Patents Full-Text Database  
☐ US Pre-Grant Publication Full-Text Database  
☐ JPO Abstracts Database  
☐ EPO Abstracts Database  
☒ Derwent World Patents Index  
☐ IBM Technical Disclosure Bulletins

**Database:**

wo89/12694.ptpn.

**Refine Search:****Clear****Search History****Today's Date: 10/19/2001**

<u>DB Name</u>	<u>Query</u>	<u>Hit Count</u>	<u>Set Name</u>
DWPI	wo89/12694.ptpn.	0	<u>L7</u>
USPT,PGPB,JPAB,EPAB,DWPI	wo89/12694.ptpn.	1	<u>L6</u>
USPT,PGPB,JPAB,EPAB,DWPI	(secondary adj structure) and (loop same (double adj stranded))	266	<u>L5</u>
USPT,PGPB,JPAB,EPAB,DWPI	loop same (double adj stranded)	829	<u>L4</u>
USPT,PGPB,JPAB,EPAB,DWPI	(loop same (double adj stranded))	829	<u>L3</u>
USPT,PGPB,JPAB,EPAB,DWPI	((secondary adj structure) same (loop and (double adj stranded)))	131	<u>L2</u>
DWPI	RNA and ((secondary adj structure) same (loop and (double adj stranded)))	1	<u>L1</u>

Logging in to Dialog

Trying 3106900061...Open

DIALOG INFORMATION SERVICES

PLEASE LOGON:

\*\*\*\*\*

ENTER PASSWORD:

□t840lcpq

\*\*\*\*\*

Welcome to DIALOG

Dialog leel 01.10.01D

Lat logoff: 09oct01 14:58:21

Logon file001 24oct01 13:33:19

\*\*\* ANNOUNCEMENT \*\*\*

\*\*\*

--Important Notice to Freelance Athor--

See HELP FREELANCE for more information

\*\*\*

NEW FILES RELEASED

\*\*\*Diclore Databae (File 101)

\*\*\*Harri Bine Profiler (File 537)

\*\*\*Mergent Compan Profile (File 555)

\*\*\*Mergent Compan Snaophot (File 556)

\*\*\*Mergent Compan New Report (File 557)

\*\*\*Financial Time Flltext (File 476)

\*\*\*TRADEMARKSCAN-Japan (File 669)

UPDATING RESUMED

\*\*\*Delphe Eropean Bine (File 481)

\*\*\*Book In Print (File 470)

\*\*\*

RELOADED

\*\*\*CLAIMS/US PATENTS (File 340, 341, 942)

\*\*\*Kompa Middle Eat/Africa/Mediterranean (File 585)

\*\*\*Kompa Aia/Pacific (File 592)

\*\*\*Kompa Central/Eatern Erope (File 593)

\*\*\*Kompa Canada (File 594)

\*\*\*CANCERLIT (File 159)

\*\*\*Information Science Abtract (File 202)

\*\*\*New docment pplier\*\*\*

IMED ha been changed to INFOTRIE (ee HELP OINFOTRI)

>>>Get immediate new with Dialog' Firt Releae  
new erice. Firt Releae pdate major newwire  
databae within 15 minte of tranmiion oer the  
wire. Firt Releae proide fll Dialog earchabilit  
and fll-text featre. To earch Firt Releae file in  
OneSearch impl BEGIN FIRST for coerage from Dialog'  
broad pectrm of new wire.

>>> Enter BEGIN HOMEBASE for Dialog Annoncement <<<

>>> of new databae, price change, etc. <<<

\*\*\*\*

File 1:ERIC 1966-2001/Oct 04

(c) format onl 2001 The Dialog Corporation

Set Item Decription

--- -----

Ecker et al

Cot i in DialUnit  
? dialog

>>>'IALOG' not recognized as set or accession number  
? b 410

24oct01 13:33:22 User233835 Session D513.1  
\$0.50 0.143 DialUnits File1  
\$0.50 Estimated cost File1  
\$0.05 TYMNET  
\$0.55 Estimated cost this search  
\$0.55 Estimated total session cost 0.143 DialUnits

File 410:Chronolog(R) 1981-2001/Oct  
(c) 2001 The Dialog Corporation

Set	Items	Description
-----	-------	-------------

---	-----	-----
-----	-------	-------

? set hi ;set hi

HILIGHT set on as ''

**HILIGHT set on as ''**

? b 155, 5, 357, 73

24oct01 13:33:34 User233835 Session D513.2  
\$0.00 0.064 DialUnits File410  
\$0.00 Estimated cost File410  
\$0.01 TYMNET  
\$0.01 Estimated cost this search  
\$0.56 Estimated total session cost 0.207 DialUnits

SYSTEM:OS - DIALOG OneSearch

File 155:MEDLINE(R) 1966-2001/Nov W3

File 5:Biosis Previews(R) 1969-2001/Oct W3

(c) 2001 BIOSIS

File 357:Derwent Biotechnology Abs 1982-2001/Dec B1

(c) 2001 Derwent Publ Ltd

\*File 357: Price changes as of 1/1/01. Please see HELP RATES 357.

File 73:EMBASE 1974-2001/Oct W2

(c) 2001 Elsevier Science B.V.

\*File 73: For information about Explode feature please  
see Help News73.

Set	Items	Description
-----	-------	-------------

---	-----	-----
-----	-------	-------

? s loop and ((double (w) stranded) or stem)

121850	LOOP
520065	DOUBLE
92974	STRANDED
45698	DOUBLE(W) STRANDED
271009	STEM

S1	10500	LOOP AND ((DOUBLE (W) STRANDED) OR STEM)
----	-------	--

? s s1 and RNA

10500	S1	
900435	RNA	
S2	7314	S1 AND RNA

? s s2 and interleukin

7314	S2	
348829	INTERLEUKIN	
S3	38	S2 AND INTERLEUKIN

? rd

...completed examining records

S4	27	RD (unique items)
----	----	-------------------

? 't s4/6/1-27

4/6/1 (Item 1 from file: 155)  
09989538 99058018 PMID: 9838089

Alternative splicing of mouse IL-15 is due to the use of an internal splice site in exon 5.

Dec 10 1998

4/6/2 (Item 2 from file: 155)  
09621693 98049231 PMID: 9389363

**Interleukin-9** in human myeloid leukemia cells.

Aug 1997

4/6/3 (Item 3 from file: 155)  
09529425 97313464 PMID: 9169458

HIV-1 Tat induces the expression of the **interleukin-6** (IL6) gene by binding to the IL6 leader **RNA** and by interacting with CAAT enhancer-binding protein beta (NF-IL6) transcription factors.

Jun 6 1997

4/6/4 (Item 4 from file: 155)  
09281337 97223466 PMID: 9070287

A minimised hammerhead ribozyme with activity against **interleukin-2** in human cells.

Feb 13 1997

4/6/5 (Item 5 from file: 155)  
09269258 97211760 PMID: 9058727

CD30 ligand is frequently expressed in human hematopoietic malignancies of myeloid and lymphoid origin.

Mar 15 1997

4/6/6 (Item 6 from file: 155)  
09181168 96430277 PMID: 8833403

C-kit ligand (SCF) in human multiple myeloma cells.

Feb 1996

4/6/7 (Item 7 from file: 155)  
09172502 96354215 PMID: 8761952

Expression of basic helix-**loop**-helix transcription factors in explant hematopoietic progenitors.

Jun 1 1996

4/6/8 (Item 8 from file: 155)  
09088756 97098461 PMID: 8943001

A cytokine mRNA-destabilizing element that is structurally and functionally distinct from A+U-rich elements.

Nov 26 1996

4/6/9 (Item 9 from file: 155)  
08924337 96260074 PMID: 8661203

Transgenic animals demonstrate a role for the IL-1 receptor in regulating IL-1beta gene expression at steady-state and during the systemic stress induced by acute pancreatitis.

Jun 1996

4/6/10 (Item 10 from file: 155)

08866964 96184798 PMID: 86 5

Plasma cells induce apoptosis of pre-B cells by interacting with bone marrow stromal cells.

Apr 15 1996

4/6/11 (Item 11 from file: 155)

08425692 94229206 PMID: 7513651

A murine stromal cell line promotes the proliferation of the human factor-dependent leukemic cell line UT-7.

May 1994

4/6/12 (Item 12 from file: 155)

08182737 94286556 PMID: 8016095

The E2A and tal-1 helix-loop-helix proteins associate in vivo and are modulated by Id proteins during interleukin 6-induced myeloid differentiation.

Jun 21 1994

4/6/13 (Item 13 from file: 155)

08071017 93195347 PMID: 8450221

Platelet-derived growth factor (PDGF) activates primitive hematopoietic precursors (pre-CFCmulti) by up-regulating IL-1 in PDGF receptor-expressing macrophages.

Mar 15 1993

4/6/14 (Item 14 from file: 155)

05913867 88156918 PMID: 3258060

Autocrine generation and requirement of BSF-2/IL-6 for human multiple myelomas.

Mar 3 1988

4/6/15 (Item 15 from file: 155)

05594657 86205871 PMID: 3085085

Identification of a positive retroregulator that stabilizes mRNAs in bacteria.

May 1986

4/6/16 (Item 1 from file: 5)

12760704 BIOSIS NO.: 200000514327

p56dck-2 as a cytokine-inducible inhibitor of cell proliferation and signal transduction.

2000

4/6/17 (Item 2 from file: 5)

11647274 BIOSIS NO.: 199800429005

Transcription factor SCL is required for c-kit expression and c-kit function in hemopoietic cells.

1998

4/6/18 (Item 3 from file: 5)

08986935 BIOSIS NO.: 199396138436

Progenitor cell hyperplasia with rare development of myeloid leukemia in interleukin 11 bone marrow chimeras.

1993

4/6/19 (Item 4 from file: 5)

08832233 BIOSIS NO.: 199395121584

Human stem cell factor (c-kit ligand) induces an autocrine loop

of growth in a GM-CSF-depend megakaryocytic leukemia cell line.  
1993

4/6/20 (Item 5 from file: 5)  
05203177 BIOSIS NO.: 000082043799  
IDENTIFICATION OF A POSITIVE RETROREGULATOR THAT STABILIZES MESSENGER  
RNA SPECIES IN BACTERIA  
1986

4/6/21 (Item 1 from file: 357)  
0047839 DBA Accession No.: 86-05687  
New positive retroregulatory element - useful for ligation downstream of  
DNA sequence expressionable for gene product to enhance expression  
1996

4/6/22 (Item 1 from file: 73)  
11285555 EMBASE No: 2001295261  
Double-stranded RNA regulates IL-4 expression  
01 SEP 2001

4/6/23 (Item 2 from file: 73)  
10751725 EMBASE No: 2000231911  
Translational pathophysiology: A novel molecular mechanism of human  
disease  
01 JUN 2000

4/6/24 (Item 3 from file: 73)  
06389337 EMBASE No: 1996038191  
Regulation of murine macrophage IL-12 production: Activation of  
macrophages in vivo, restimulation in vitro, and modulation by other  
cytokines  
1996

4/6/25 (Item 4 from file: 73)  
06040056 EMBASE No: 1995070323  
Cell-to-cell interaction of cytokine-dependent myeloblastic line  
constitutively expressing membrane-bound stem cell factor abrogates  
cytokine dependency partially through granulocyte-macrophage  
colony-stimulating factor production  
1995

4/6/26 (Item 5 from file: 73)  
05481966 EMBASE No: 1993250065  
The SCL gene product is regulated by and differentially regulates  
cytokine responses during myeloid leukemic cell differentiation  
1993

4/6/27 (Item 6 from file: 73)  
04901544 EMBASE No: 1992041759  
Ferritin gene expression in health and malignancy  
1992  
? s loop and (double (w) stranded)

121850 LOOP  
520065 DOUBLE  
92974 STRANDED  
45698 DOUBLE(W) STRANDED

S5 1243 LOOP AND (DOUBLE (W) STRANDED)  
? s s5 and RNA

1243 S5  
900435 RNA  
S6 685 S5 AND RNA  
? s s6 and interleukin

685 S6  
348829 INTERLEUKIN  
S7 2 S6 AND INTERLEUKIN  
? rd

...completed examining records  
S8 2 RD (unique items)  
? t s8/6/1-2

8/6/1 (Item 1 from file: 73)  
11285555 EMBASE No: 2001295261  
**Double-stranded RNA** regulates IL-4 expression  
01 SEP 2001

8/6/2 (Item 2 from file: 73)  
06389337 EMBASE No: 1996038191  
Regulation of murine macrophage IL-12 production: Activation of  
macrophages in vivo, restimulation in vitro, and modulation by other  
cytokines  
1996  
? t s8/7/1-2

8/7/1 (Item 1 from file: 73)  
DIALOG(R)File 73:EMBASE  
(c) 2001 Elsevier Science B.V. All rts. reserv.

11285555 EMBASE No: 2001295261  
**Double-stranded RNA** regulates IL-4 expression  
Kehoe K.E.; Brown M.A.; Imani F.  
Dr. F. Imani, Division of Clinical Immunology, Department of Medicine,  
Johns Hopkins Univ. Sch. of Medicine, 5501 Hopkins Bayview Circle,  
Baltimore, MD 21224 United States  
AUTHOR EMAIL: fimani@mail.jhmi.edu  
Journal of Immunology ( J. IMMUNOL. ) (United States) 01 SEP 2001,  
167/5 (2496-2501)  
CODEN: JOIMA ISSN: 0022-1767  
DOCUMENT TYPE: Journal ; Article  
LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH  
NUMBER OF REFERENCES: 39

dsRNA, as genomic fragment, replicative intermediate, or stem and  
**loop** structure in cells infected by viruses, can act to signal the  
immune system of the presence of viral infections. Although most viral  
infections are associated with strong Th1 immune responses, Th2-type  
responses have also been observed. In this study, we characterize the  
effects of dsRNA on the induction of Th2 responses in human lymphocytes. We  
report that in addition to the well-known Th1-inducing capabilities of  
dsRNA, treatment of human lymphocytes with low concentrations of dsRNA  
(0.1-1 mug/ml) leads to the expression of the prototypic Th2 cytokine IL-4.  
This induction was accompanied with the concentration-dependent activation  
of NF-kappaB and NF-AT2 but not NF-AT1. In addition, dsRNA can directly  
activate an IL-4 promoter-driven chloramphenicol acetyltransferase reporter  
gene in transiently transfected Jurkat cells. These results are the first  
demonstration of a non-TCR-associated activator of NF-AT in human cells and  
suggest that dsRNA directly influences IL-4 gene expression through its  
effect on NF-AT activation. Our data provide support for the idea that  
dsRNA at low concentrations in vivo may induce a Th2-dominant response that  
is not optimal for protective immunity to the virus.



8/7/2 (Item 2 from file: 73)  
DIALOG(R)File 73:EMBASE  
(c) 2001 Elsevier Science B.V. All rts. reserv.

06389337 EMBASE No: 1996038191

Regulation of murine macrophage IL-12 production: Activation of  
macrophages in vivo, restimulation in vitro, and modulation by other  
cytokines

Skeen M.J.; Miller M.A.; Shinnick T.M.; Ziegler H.K.

Dept. of Microbiology and Immunology, Rollins Research Center, Emory  
University, Atlanta, GA 30322 United States

Journal of Immunology ( J. IMMUNOL. ) (United States) 1996, 156/3  
(1196-1206)

CODEN: JOIMA ISSN: 0022-1767

DOCUMENT TYPE: Journal; Article

LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

IL-12 is important in the host response to a variety of pathogens. It plays an adjuvant-like role in an initial immune response as well as a therapeutic role in established infections. Despite its well documented importance, comparatively little is known about the regulation of IL-12 production. In this study, we examined IL-12 production by cultured murine peritoneal macrophages from two perspectives: 1) macrophage activation in vivo, and 2) stimulation of IL-12 secretion in vitro. Macrophages were maximally activated within 48 h in vivo during infection with *Listeria*. Interestingly, although avirulent or heat-killed *Listeria* induced only minimal production of IL-12 by macrophages, the immunogenic combination of heat-killed bacteria and rIL-12 was highly stimulatory for IL-12 production. LPS and peritoneal inflammatory agents were also stimulatory, but latex beads were ineffective, indicating that microbial components were essential and phagocytosis alone was insufficient. Restimulation in vitro revealed similar patterns, in that infection and LPS were stimulatory but latex beads were not. A systematic survey of potential stimulatory agents showed that microbial heat shock proteins, crude bacterial extracts, bacterial superantigens, a yeast extract, and dsRNA induced IL-12 in vitro. Other cytokines also influenced IL-12 induction. IFN-gamma, which is up-regulated during infection, acted in synergy with other stimuli, suggesting an amplification **loop** for IL-12 production, whereas IL-4, IL-10, IL-13, and TGF- beta were inhibitory. The existence of a broad range of stimuli from a wide variety of pathogenic organisms underscores the fundamental importance of IL- 12 in host defense.

? ds

Set	Items	Description
S1	10500	LOOP AND ((DOUBLE (W) STRANDED) OR STEM)
S2	7314	S1 AND RNA
S3	38	S2 AND INTERLEUKIN
S4	27	RD (unique items)
S5	1243	LOOP AND (DOUBLE (W) STRANDED)
S6	685	S5 AND RNA
S7	2	S6 AND INTERLEUKIN
S8	2	RD (unique items)

? s s5 and interleukin

	1243	S5
	348829	INTERLEUKIN
S9	6	S5 AND INTERLEUKIN

? rd

...completed examining records

S10	4	RD (unique items)
-----	---	-------------------

? t s10/6/1-4

10/6/1 (Item 1 from file: 356)  
09197808 97136883 PMID: 8982260

Peptide nucleic acids directed to the promoter of the alpha-chain of the **interleukin-2** receptor.

Dec 11 1996

10/6/2 (Item 1 from file: 357)  
0016754 DBA Accession No.: 84-00029

Cloning of **double stranded** cDNA from major and minor components of mRNA - role of reverse-transcriptase review e.g. Pseudopleuronectes americanus antifreeze and T leukocyte **interleukin 2** cDNA cloning (conference paper) 1982

10/6/3 (Item 1 from file: 73)  
11285555 EMBASE No: 2001295261  
**Double-stranded** RNA regulates IL-4 expression  
01 SEP 2001

10/6/4 (Item 2 from file: 73)  
06389337 EMBASE No: 1996038191  
Regulation of murine macrophage IL-12 production: Activation of macrophages in vivo, restimulation in vitro, and modulation by other cytokines  
1996  
? t s10/7/1-4

10/7/1 (Item 1 from file: 155)  
DIALOG(R) File 155:MEDLINE(R)

09197808 97136883 PMID: 8982260

Peptide nucleic acids directed to the promoter of the alpha-chain of the **interleukin-2** receptor.

Praseuth D; Grigoriev M; Guieysse AL; Pritchard LL; Harel-Bellan A; Nielsen PE; Helene C

Laboratoire de Biophysique, INSERM U.201-CNRS URA 481, Museum National d'Histoire Naturelle, Paris, France. praseuth@mnhn.fr

Biochimica et biophysica acta (NETHERLANDS) Dec 11 1996, 1309 (3) p226-38, ISSN 0006-3002 Journal Code: AOW

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Two 10-mer oligopyrimidine peptide nucleic acids (PNAs) were designed to interfere with IL-2R alpha promoter expression by binding to the regulatory sequences overlapping SRF and NF-kappa B transcription factor sites. Specific complexes were formed on each target sequence, and clearly involved (1) Hoogsteen hydrogen bonds as shown by experiments in which the purine strand of a single or **double-stranded** target was substituted with 7-deazadeoxyguanosine, (2) P-loop formation on double-helical DNA as evidenced by susceptibility to a single-strand-specific nuclease. When formed on a single-stranded DNA target, these highly stable complexes were responsible for efficient physical blockage of T7 DNA polymerase elongation on the template DNA containing the target oligopurine sequence. On a **double-stranded** target, these complexes only formed at low ionic strength and were slowly dissociated at physiological ionic strength (pH 6.5) with a t1/2 of 6.5-7 h. The salt-dependent instability of preformed complexes on a plasmid target was probably the critical factor responsible for their lack of significant sequence-specific effect on IL-2R alpha promoter activity inside living cells.

Record Date Created: 19970122

10/7/2 (Item 1 from file: 357)

DIALOG(R) File 357:Derwent Biotology Abs  
(c) 2001 Derwent Publ Ltd. All rts. reserv.

0016754 DBA Accession No.: 84-00029

Cloning of **double stranded** cDNA from major and minor components  
of mRNA - role of reverse-transcriptase review e.g. Pseudopleuronectus  
americanus antifreeze and T leukocyte **interleukin 2** cDNA cloning  
(conference paper)

AUTHOR: Lin Y

CORPORATE SOURCE: Biological Carcinogenesis Program, National Cancer  
Institute-Frederick Cancer Research Facility, Frederick, Maryland, USA.  
(111-27) 1982

CODEN: 9999Z

LANGUAGE: English

ABSTRACT: Mature mRNA specific for certain proteins is found in the  
cytoplasm where it actively synthesizes these proteins. Methods of  
isolating and purifying poly(A)-mRNA are briefly discussed. In the  
synthesis of single-stranded (ss)-DNA from mRNA, reverse-transcriptase  
is used for ss-DNA synthesis; mRNA is dissociated from the cDNA-mRNA  
complex and the enzyme is denatured by boiling; the mixture is  
centrifuged ; DNA polymerase I is added to the supernatant for the  
synthesis of **double-stranded** (ds)-DNA. The hair-pin  
**loop** appearing by chance on the ss-cDNA and serving as primer for  
ds-DNA synthesis, is removed to give an open-ended ds-cDNA used for  
insertion into a plasmid. The hybrid plasmid can then be used to  
transform a host organism such as Escherichia coli. The application of  
these techniques in the cloning of antifreeze cDNA from  
Pseudopleuronectus americanus and **interleukin 2** cDNA from monkey  
T leukocytes is described. New cloning vectors and cloning procedures  
are discussed. (41 ref)

10/7/3 (Item 1 from file: 73)

DIALOG(R) File 73:EMBASE

(c) 2001 Elsevier Science B.V. All rts. reserv.

11285555 EMBASE No: 2001295261

**Double-stranded** RNA regulates IL-4 expression

Kehoe K.E.; Brown M.A.; Imani F.

Dr. F. Imani, Division of Clinical Immunology, Department of Medicine,  
Johns Hopkins Univ. Sch. of Medicine, 5501 Hopkins Bayview Circle,  
Baltimore, MD 21224 United States

AUTHOR EMAIL: fimani@mail.jhmi.edu

Journal of Immunology ( J. IMMUNOL. ) (United States) 01 SEP 2001,  
167/5 (2496-2501)

CODEN: JOIMA ISSN: 0022-1767

DOCUMENT TYPE: Journal ; Article

LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

NUMBER OF REFERENCES: 39

dsRNA, as genomic fragment, replicative intermediate, or stem and  
**loop** structure in cells infected by viruses, can act to signal the  
immune system of the presence of viral infections. Although most viral  
infections are associated with strong Th1 immune responses, Th2-type  
responses have also been observed. In this study, we characterize the  
effects of dsRNA on the induction of Th2 responses in human lymphocytes. We  
report that in addition to the well-known Th1-inducing capabilities of  
dsRNA, treatment of human lymphocytes with low concentrations of dsRNA  
(0.1-1 mug/ml) leads to the expression of the prototypic Th2 cytokine IL-4.  
This induction was accompanied with the concentration-dependent activation  
of NF-kappaB and NF-AT2 but not NF-AT1. In addition, dsRNA can directly  
activate an IL-4 promoter-driven chloramphenicol acetyltransferase reporter  
gene in transiently transfected Jurkat cells. These results are the first  
demonstration of a non-TCR-associated activator of NF-AT in human cells and  
suggest that dsRNA directly influences IL-4 gene expression through its  
effect on NF-AT activation. Our data provide support for the idea that  
dsRNA at low concentrations in vivo may induce a Th2-dominant response that

is not optimal for protective immunity to the virus.

10/7/4 (Item 2 from file: 73)  
DIALOG(R) File 73:EMBASE  
(c) 2001 Elsevier Science B.V. All rts. reserv.

06389337 EMBASE No: 1996038191

Regulation of murine macrophage IL-12 production: Activation of macrophages in vivo, restimulation in vitro, and modulation by other cytokines

Skeen M.J.; Miller M.A.; Shinnick T.M.; Ziegler H.K.

Dept. of Microbiology and Immunology, Rollins Research Center, Emory University, Atlanta, GA 30322 United States

Journal of Immunology ( J. IMMUNOL. ) (United States) 1996, 156/3 (1196-1206)

CODEN: JOIMA ISSN: 0022-1767

DOCUMENT TYPE: Journal; Article

LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

IL-12 is important in the host response to a variety of pathogens. It plays an adjuvant-like role in an initial immune response as well as a therapeutic role in established infections. Despite its well documented importance, comparatively little is known about the regulation of IL-12 production. In this study, we examined IL-12 production by cultured murine peritoneal macrophages from two perspectives: 1) macrophage activation in vivo, and 2) stimulation of IL-12 secretion in vitro. Macrophages were maximally activated within 48 h in vivo during infection with *Listeria*. Interestingly, although avirulent or heat-killed *Listeria* induced only minimal production of IL-12 by macrophages, the immunogenic combination of heat-killed bacteria and rIL-12 was highly stimulatory for IL-12 production. LPS and peritoneal inflammatory agents were also stimulatory, but latex beads were ineffective, indicating that microbial components were essential and phagocytosis alone was insufficient. Restimulation in vitro revealed similar patterns, in that infection and LPS were stimulatory but latex beads were not. A systematic survey of potential stimulatory agents showed that microbial heat shock proteins, crude bacterial extracts, bacterial superantigens, a yeast extract, and dsRNA induced IL-12 in vitro. Other cytokines also influenced IL-12 induction. IFN-gamma, which is up-regulated during infection, acted in synergy with other stimuli, suggesting an amplification loop for IL-12 production, whereas IL-4, IL-10, IL-13, and TGF-beta were inhibitory. The existence of a broad range of stimuli from a wide variety of pathogenic organisms underscores the fundamental importance of IL-12 in host defense.

? ds

Set	Items	Description
S1	10500	LOOP AND ((DOUBLE (W) STRANDED) OR STEM)
S2	7314	S1 AND RNA
S3	38	S2 AND INTERLEUKIN
S4	27	RD (unique items)
S5	1243	LOOP AND (DOUBLE (W) STRANDED)
S6	685	S5 AND RNA
S7	2	S6 AND INTERLEUKIN
S8	2	RD (unique items)
S9	6	S5 AND INTERLEUKIN
S10	4	RD (unique items)

? t s4/7/4-8, 10-12, 21, 23, 27

4/7/4 (Item 4 from file: 155)  
DIALOG(R) File 155:MEDLINE(R)

09281337 97223466 PMID: 9070287

A minimised hammerhead ribozyme with activity against interleukin-2 in human cells.

Sioud M; Opstad A; Hendry P; Eckett TJ; Jennings PA; McCall  
Institute of Immunology and Rheumatology, National Hospital, Oslo,  
Norway.

Biochemical and biophysical research communications (UNITED STATES) Feb  
13 1997, 231 (2) p397-402, ISSN 0006-291X Journal Code: 9Y8

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

A "minizyme" is a smaller version of the hammerhead ribozyme, in which **stem-loop II** has been replaced by a short linker. Here, we have synthesised a DNA-containing minizyme and a ribozyme, which are designed to cut within a 15-nucleotide sequence in human **interleukin-2** mRNA, and have tested for their activity in vitro and in cells. In vitro at 37 degrees C, a minizyme with linker of sequence d(GTTTT) cleaves a 15-ribonucleotide synthetic substrate 5-fold slower than does the full-sized ribozyme. In human cells, the minizyme inhibits the production of **interleukin -2** protein to a similar extent as does the ribozyme. Also, the minizyme and the ribozyme are more effective in cells than any of three controls: an inactive minizyme, a 15-nucleotide antisense DNA, or DNA of random sequence. The positive effect observed in cells indicates that minizymes may be useful as pharmaceuticals.

Record Date Created: 19970417

4/7/5 (Item 5 from file: 155)  
DIALOG(R) File 155:MEDLINE(R)

09269258 97211760 PMID: 9058727

CD30 ligand is frequently expressed in human hematopoietic malignancies of myeloid and lymphoid origin.

Gattei V; Degan M; Gloghini A; De Iuliis A; Improta S; Rossi FM; Aldinucci D; Perin V; Serraino D; Babare R; Zagonel V; Gruss HJ; Carbone A; Pinto A

Department of Medical Oncology, Centro di Riferimento Oncologico, INRCCS, Aviano, Italy.

Blood (UNITED STATES) Mar 15 1997, 89 (6) p2048-59, ISSN 0006-4971  
Journal Code: A8G

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

CD30 ligand (CD30L) is a type-II membrane glycoprotein capable of transducing signals leading to either cell death or proliferation through its specific counterstructure CD30. Although several lines of evidence indicate that CD30L plays a key role as a paracrine- or autocrine-acting surface molecule in the deregulated cytokine cascade of Hodgkin's disease, little is known regarding its distribution and biologic significance in other human hematopoietic malignancies. By analyzing tumor cells from 181 patients with **RNA** studies and immunostaining by the anti-CD30L monoclonal antibody M80, we were able to show that human hematopoietic malignancies of different lineage and maturation stage display a frequent and broad expression of the ligand. CD30L mRNA and surface protein were detected in 60% of acute myeloid leukemias (AMLs), 54% of B-lineage acute lymphoblastic leukemias (ALLs), and in a consistent fraction (68%) of B-cell lymphoproliferative disorders. In this latter group, hairy cell leukemia and high-grade B-cell non-Hodgkin's lymphoma (B-NHL) expressed a higher surface density of CD30L as compared with B-cell chronic lymphocytic leukemia and low-grade B-NHL. Purified plasmacells from a fraction of multiple myeloma patients also displayed CD30L mRNA and protein. A more restricted expression of CD30L was found in T-cell tumors that was mainly confined to neoplasms with an activated peripheral T-cell phenotype, such as T-cell prolymphocytic leukemia, peripheral T-NHL, and adult T-cell leukemia/lymphoma. In contrast, none of the T-lineage ALLs analyzed expressed the ligand. In AML, a high cellular density of CD30L was detected in French-American-British M3, M4, and M5 phenotypes, which are directly associated with the presence on tumor cells of certain surface structures, including the p55 **interleukin-2** receptor alpha-chain, the alpha(M) (CD11b) chain of beta2 integrins, and the intercellular adhesion molecule-1

(CD54). Analysis of normal hematopoietic cells evidenced that, addition to circulating and tonsil B cells, a fraction of bone marrow myeloid precursors, erythroblasts, and subsets of megakaryocytes also express CD30L. Finally, we have shown that native CD30L expressed on primary leukemic cells is functionally active by triggering both mitogenic and antiproliferative signals on CD30+ target cells. As opposed to CD30L, only 10 of 181 primary tumors expressed CD30 mRNA or protein, rendering therefore unlikely a CD30-CD30L autocrine loop in human hematopoietic neoplasms. Taken together, our data indicate that CD30L is widely expressed from early to late stages of human hematopoiesis and suggest a regulatory role for this molecule in the interactions of normal and malignant hematopoietic cells with CD30+ immune effectors and/or microenvironmental accessory cells.

Record Date Created: 19970402

4/7/6 (Item 6 from file: 155)  
DIALOG(R) File 155:MEDLINE(R)

09181168 96430277 PMID: 8833403

C-kit ligand (SCF) in human multiple myeloma cells.

Lemoli RM; Fortuna A

Institute of Hematology, University of Bologna, Italy.

Leukemia & lymphoma (SWITZERLAND) Feb 1996, 20 (5-6) p457-64, ISSN 1042-8194 Journal Code: BNQ

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

Here we review our recent experience addressing the role of SCF in multiple myeloma (MM). We first investigated the proliferation of MM cell lines and bone marrow samples from myeloma patients in response to rh-SCF alone and combined with **Interleukin-6** (IL-6), IL-3, and IL-3/GM-CSF fusion protein PIXY 321. Neoplastic plasma cells were highly purified (>90%) by immunomagnetic depletion of T, myeloid, monocytoid and NK cells. The number of S-phase cells was evaluated after 3 days of liquid culture by the bromodeoxyuridine (BRDU) incorporation assay. The proliferation of RPMI 8226 and U266 cell lines was also assessed by a clonogenic assay. All the experiments were performed in serum-free conditions. RPMI 8226 cell line was not stimulated by SCF which also did not augment the proliferative activity of IL-6, IL-3 and PIXY-321. Conversely, SCF addition resulted in 2.4-fold increase of the number of U266 colonies and in a higher number of U266 and MT3 cells in S-phase. The c-kit ligand also enhanced the proliferation of MT3 and U266 cells mediated by the other cytokines. Anti-SCF polyclonal antibodies completely abrogated the proliferative response of MT3 cells to exogenous SCF and markedly reduced the spontaneous growth of the same cell line. Reverse transcriptase-polymerase chain reaction amplification (RT-PCR) did detect SCF mRNA in MT3 and RPMI 8226 cells. Moreover, secreted SCF was found, in a biologically active form, in the supernatant of the two cell lines by the MO7e proliferation assay. These results suggest that an autocrine proliferative **loop** may be operative in MT3 cell line. When tested on fresh myeloma samples, SCF increased the number of S-phase plasma cells (4.7 +/- 1.6% vs 3.4 +/- 1.3% in control cultures; p = 0.02). Significant proliferation was also induced by IL6, IL-3 and PIXY-321. The addition of SCF significantly enhanced the proliferation of myeloma cells responsive to IL-6. Preliminary experiments performed on circulating plasma cells and myeloma precursors further supported the role of SCF on the proliferation of the neoplastic clone in MM.

Record Date Created: 19970612

4/7/7 (Item 7 from file: 155)  
DIALOG(R) File 155:MEDLINE(R)

09172502 96354215 PMID: 8761952

Expression of basic helix-loop-helix transcription factors in explant hematopoietic progenitors.

Quesenberry PJ; Iscove NN; Cooper C; Brady G; Newburger P tein GS;  
Stein JS; Reddy GP; Pearson-White S  
Cancer Center, University of Massachusetts Medical Center, Worcester  
01605, USA.

Journal of cellular biochemistry (UNITED STATES) Jun 1 1996, 61 (3)  
p478-88, ISSN 0730-2312 Journal Code: HNF  
Contract/Grant No.: 5R01 CA27466-17, CA, NCI  
Languages: ENGLISH  
Document type: Journal Article  
Record type: Completed

The basic helix-loop-helix (bHLH) transcription factors form heterodimers and control steps in cellular differentiation. We have studied four bHLH transcription factors, SCL, lyl-1, E12/E47, and Id-1, in individual lineage-defined progenitors and hematopoietic growth factor-dependent cell lines, evaluating mRNA expression and the effects of growth factors and cell cycle phase on this expression. Single lineage-defined progenitors selected from early murine colony starts and grown under permissive conditions were analyzed by RT-PCR. SCL and E12/E47 were expressed in the vast majority of tri-, bi-, and unilineage progenitors of erythroid, macrophage, megakaryocyte, and neutrophil lineages. Expression for E12/E47 was not seen in unilineage megakaryocyte and erythroid or bilineage neutrophil/mast cell progenitors. Lyl-1 showed a more restricted pattern of expression, although expression was seen in some bi- and unilineage progenitors. No expression was detected in erythroid, erythroid-megakaryocyte-macrophage, macrophage-neutrophil, macrophage, or megakaryocytic progenitors. Id-1, an inhibitory bHLH transcription factor, was also widely expressed in all bi- and unilineage progenitors; only the trilineage erythroid-megakaryocyte-macrophage progenitors failed to show expression. Expression of these factors within a progenitor class was generally heterogeneous, with some progenitors showing expression and some not. This was seen even when two sister cells from the same colony start were analyzed. Id-1, but not E12/E47, mRNA was increased in FDC-P1 and MO7E hematopoietic cell lines after exposure to IL-3 or GM-CSF. Id-1, E12, and lyl-1 showed marked variation at different points in cell cycle in isoleucine-synchronized FDC-P1 cells. These results suggest that SCL, lyl-1, E12/E47, and Id-1 are important in hematopoietic progenitor cell regulation, and that their expression in hematopoietic cells varies in response to cytokines and/or during transit through cell cycle.

Record Date Created: 19970429

4/7/8 (Item 8 from file: 155)  
DIALOG(R)File 155:MEDLINE(R)

09088756 97098461 PMID: 8943001

A cytokine mRNA-destabilizing element that is structurally and functionally distinct from A+U-rich elements.

Brown CY; Lagnado CA; Goodall GJ

Hanson Centre for Cancer Research, Institute of Medical and Veterinary Science, Adelaide, Australia.

Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Nov 26 1996, 93 (24) p13721-5, ISSN 0027-8424  
Journal Code: PV3

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

The control of mRNA stability is crucial to the regulation of cytokine expression. We describe here a novel, potent destabilizing element found in the 3' untranslated region of granulocyte colony-stimulating factor mRNA. This element, which appears to require at least one **stem-loop** structure, we term the **stem-loop** destabilizing element (SLDE). Functionally equivalent elements appear to also exist in the **interleukin 2** and **interleukin 6** mRNAs. The SLDE is functionally distinct from the A+U-rich elements, which are also present in these and other cytokine mRNAs, because it destabilizes a chimeric mRNA in a tumor cell line in which A+U-rich elements do not function. In addition, the effect of the SLDE is insensitive to calcium ionophore and is therefore

regulated independently of A+U stabilizing elements. The existence of two distinct mRNA-stabilizing elements provides an additional mechanism for the differential regulation of cytokine expression.

Record Date Created: 19970116

4/7/10 (Item 10 from file: 155)  
DIALOG(R) File 155:MEDLINE(R)

08866964 96184798 PMID: 8605355

Plasma cells induce apoptosis of pre-B cells by interacting with bone marrow stromal cells.

Tsujimoto T; Lisukov IA; Huang N; Mahmoud MS; Kawano MM

Department of Hematology and Oncology, Research Institute for Radiation Biology and Medicine, Hiroshima University, Japan.

Blood (UNITED STATES) Apr 15 1996, 87 (8) p3375-83, ISSN 0006-4971  
Journal Code: A8G

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

By using two-color phenotypic analysis with fluorescein isothiocyanate-anti-CD38 and phycoerythrin-anti-CD19 antibodies, we found that pre-B cells (CD38+CD19+) significantly decreased depending on the number of plasma cells (CD38++CD19+) in the bone marrow (BM) in the cases with BM plasmacytosis, such as myelomas and even polyclonal gammopathy. To clarify how plasma cells suppress survival of pre-B cells, we examined the effect of plasma cells on the survival of pre-B cells with or without BM-derived stromal cells in vitro. Pre-B cells alone rapidly entered apoptosis, but interleukin-7 (IL-7), a BM stromal cell line (KM-102), or culture supernatants of KM-102 cells could support pre-B cell survival. On the other hand, inhibitory factors such as transforming growth factor-beta1 (TGF-beta1) and macrophage inflammatory protein-1beta (MIP-1beta) could suppress survival of pre-B cells even in the presence of IL-7. Plasma cells alone could not suppress survival of pre-B cells in the presence of IL-7, but coculture of plasma cells with KM-102 cells or primary BM stromal cells induced apoptosis of pre-B cells. Supernatants of coculture with KM-102 and myeloma cell lines (KMS-5) also could suppress survival of pre-B cells. Furthermore, we examined the expression of IL-7, TGF-beta1, and MIP-1beta mRNA in KM-102 cells and primary stromal cells cocultured with myeloma cell lines (KMS-5). In these cells, IL-7 mRNA was downregulated, but the expression of TGF-beta1 and MIP-1beta mRNA was augmented. Therefore, these results suggest that BM-derived stromal cells attached to plasma (myeloma) cells were modulated to secrete lesser levels of supporting factor (IL-7) and higher levels of inhibitory factors (TGF-beta1 and MIP-1beta) for pre-B cell survival, which could explain why the increased number of plasma (myeloma) cells induced suppression of pre-B cells in the BM. This phenomenon may represent a feedback loop between pre-B cells and plasma cells via BM stromal cells in the BM.

Record Date Created: 19960517

4/7/11 (Item 11 from file: 155)  
DIALOG(R) File 155:MEDLINE(R)

08425692 94229206 PMID: 7513651

A murine stromal cell line promotes the proliferation of the human factor-dependent leukemic cell line UT-7.

Auffray I; Dubart A; Izac B; Vainchenker W; Coulombel L

INSERM U 362, Institut Gustave Roussy, Villejuif, France.

Experimental hematology (UNITED STATES) May 1994, 22 (5) p417-24,  
ISSN 0301-472X Journal Code: EPR

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

In long-term human bone marrow cultures, stromal cells of human origin are usually used on the assumption that human primitive progenitor cells do not respond to cytokines produced by stromal cells from other species.



There is accumulating evidence, however, that murine stromal cells also promote maintenance and differentiation of very primitive human stem cells, which suggests the existence of novel stromal activities that cross species barriers. In this study, we show that a murine bone marrow-derived stromal cell line, MS-5, allows the proliferation of the human leukemic cell line UT-7. The long-term growth of UT-7 is usually supported only by human interleukin-3 (IL-3), granulocyte-macrophage colony-stimulating factor (GM-CSF), or erythropoietin (Epo). None of these three cytokines was involved in the observed effect, since murine GM-CSF and IL-3 do not act on human cells and MS-5 cells do not produce Epo. Soluble stem cell factor (SCF) induced UT-7 cell proliferation. However, S1/S1 mutant fibroblasts also supported UT-7 cell growth and anti-c-kit antibodies only partially abolished UT-7 cell proliferative response to MS-5 cells. These observations excluded a major role of SCF in this system. MS-5-derived growth-promoting activity was diffusible, but attempts to grow UT-7 cells in high levels of known soluble murine stromal-derived cytokines active on human cells showed no or minimal response, suggesting that MS-5's proliferative effect was not mediated by known cytokines. Finally, involvement of an autocrine loop of activation induced by MS-5 was excluded: RT-PCR analysis did not detect increased transcripts for GM-CSF, IL-3, IL-6, SCF, or Epo in UT-7 cells cocultured for 2 to 6 days with MS-5. In addition, UT-7 cell proliferation on MS-5 was not inhibited by neutralizing antibodies against the human GM-CSF receptor or the human IL-6 receptor alpha chain. Whether UT-7 cell proliferation triggered by MS-5 reflects the existence of novel stromal cytokines or results from synergistic interactions on the MS-5 cell surface between extracellular matrix proteins and cytokines will require further investigation.

Record Date Created: 19940603

4/7/12 (Item 12 from file: 155)  
DIALOG(R) File 155:MEDLINE(R)

08182737 94286556 PMID: 8016095

The E2A and tal-1 helix-loop-helix proteins associate in vivo and are modulated by Id proteins during interleukin 6-induced myeloid differentiation.

Voronova AF; Lee F

DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA 94304-1104.

Proceedings of the National Academy of Sciences of the United States of America (UNITED STATES) Jun 21 1994, 91 (13) p5952-6, ISSN 0027-8424  
Journal Code: PV3

Languages: ENGLISH

Document type: Journal Article

Record type: Completed

The immunoglobulin enhancer-binding proteins, E12 and E47, encoded by the E2A gene belong to the basic helix-loop-helix (bHLH) family of regulatory proteins and act as transcriptional activators. In addition to their critical role in B-lymphocyte development, the E12 and E47 proteins have been implicated in the induction of myogenesis as heterodimeric partners of myogenic bHLH proteins, MyoD and myogenin. Here we demonstrate that the E2A proteins form heterodimers with the bHLH oncoprotein tal-1 in myeloid and erythroid cells and that these heterodimers specifically bind to the CANNTG DNA motif. Heterodimerization with tal-1 represses transactivation by E47 and could function to prevent the expression of immunoglobulin genes in cells other than B lymphocytes. DNA binding by E2A-tal-1 heterodimers in the M1 mouse myeloid cell line is abrogated upon terminal macrophage differentiation induced by the cytokine interleukin 6. The loss of E2A-tal-1 DNA binding is correlated with elevated expression of mRNA encoding the dominant negative HLH proteins, Id1 and particularly Id2. Moreover, recombinant Id proteins inhibit the E2A-tal-1-specific DNA binding activity from undifferentiated M1 cells. These results suggest that E2A-tal-1 heterodimers may play a role in preventing terminal differentiation in the myeloid lineage and provide a possible explanation for oncogenic transformation induced by ectopic tal-1 expression in acute T-cell lymphoblastic leukemias.

4/7/21 (Item 1 from file: 357)

DIALOG(R)File 357:Derwent Biotechnology Abs  
(c) 2001 Derwent Publ Ltd. All rts. reserv.

0047839 DBA Accession No.: 86-05687 PATENT

New positive retroregulatory element - useful for ligation downstream of  
DNA sequence expressible for gene product to enhance expression

PATENT ASSIGNEE: Cetus 1996

PATENT NUMBER: EP 174785 PATENT DATE: 960319 WPI ACCESSION NO.: 86-077173  
(8612)

PRIORITY APPLIC. NO.: US 717331 APPLIC. DATE: 850329

NATIONAL APPLIC. NO.: EP 85306183 APPLIC. DATE: 850830

LANGUAGE: English

ABSTRACT: A positive retroregulatory element which, when ligated downstream  
of a DNA sequence expressible for a gene product (I), enhances the  
expression of (I) is new. Also new is a plasmid comprising a DNA  
sequence expressible for a gene product (II) and a positive  
retroregulatory element ligated to the DNA sequence in a relationship  
to it, so that expression of (II) is enhanced. Cells transformed by the  
plasmid, and **RNA** sequences having an extended half-life conferred  
by a co-transcribed positive retroregulatory sequence are also new. The  
element preferably comprises the 3'-flanking sequence of *Bacillus*  
*thuringiensis* crystal protein gene containing an inverted repeat  
sequence. The **RNA** transcript of the inverted repeat sequence  
forms a **stem** and **loop** structure with a delta-G of -30.4  
kcal/mole. Expression of (I) coded for by the DNA sequence is  
significantly increased with the positive retroregulatory element. (I)  
And (II) are especially beta-lactamase (EC-3.5.2.6) or mammal  
**interleukin** -2, and are expressed e.g. in *Escherichia coli* or  
*Bacillus subtilis*. (65pp)

4/7/23 (Item 2 from file: 73)

DIALOG(R)File 73:EMBASE

(c) 2001 Elsevier Science B.V. All rts. reserv.

10751725 EMBASE No: 2000231911

Translational pathophysiology: A novel molecular mechanism of human  
disease

Cazzola M.; Skoda R.C.

M. Cazzola, Division of Hematology, IRCCS Policlinico S. Matteo, 27100  
Pavia Italy

AUTHOR EMAIL: m.cazzola@iol.it

Blood ( BLOOD ) (United States) 01 JUN 2000, 95/11 (3280-3288)

CODEN: BLOOA ISSN: 0006-4971

DOCUMENT TYPE: Journal; Review

LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

NUMBER OF REFERENCES: 109

In higher eukaryotes, the expression of about 1 gene in 10 is strongly  
regulated at the level of messenger **RNA** (mRNA) translation into  
protein. Negative regulatory effects are often mediated by the  
5'-untranslated region (5'-UTR) and rely on the fact that the 40S ribosomal  
subunit first binds to the cap structure at the 5'-end of mRNA and then  
scans for the first AUG codon. Self-complementary sequences can form stable  
**stem-loop** structures that interfere with the assembly of the  
preinitiation complex and/or ribosomal scanning. These **stem** loops can  
be further stabilized by the interaction with **RNA**-binding proteins,  
as in the case of ferritin. The presence of AUG codons located upstream of  
the physiological start site can inhibit translation by causing premature  
initiation and thereby preventing the ribosome from reaching the  
physiological start codon, as in the case of thrombopoietin (TPO).  
Recently, mutations that cause disease through increased or decreased  
efficiency of mRNA translation have been discovered, defining translational

pathophysiology as a novel mechanism of human disease. Hereditary hyperferritinemia/cataract syndrome arises from various point mutations or deletions within a protein-binding sequence in the 5'-UTR of the L-ferritin mRNA. Each unique mutation confers a characteristic degree of hyperferritinemia and severity of cataract in affected individuals. Hereditary thrombocythemia (sometimes called familial essential thrombocythemia or familial thrombocytosis) can be caused by mutations in upstream AUG codons in the 5'-UTR of the TPO mRNA that normally function as translational repressors. Their inactivation leads to excessive production of TPO and elevated platelet counts. Finally, predisposition to melanoma may originate from mutations that create translational repressors in the 5'-UTR of the cyclin-dependent kinase inhibitor-2A gene. (C) 2000 by The American Society of Hematology.

4/7/27 (Item 6 from file: 73)  
 DIALOG(R) File 73:EMBASE  
 (c) 2001 Elsevier Science B.V. All rts. reserv.

04901544 EMBASE No: 1992041759  
 Ferritin gene expression in health and malignancy  
 Bomford A.B.; Munro H.N.  
 USDA Human Nutrition Research, 711 Washington Street, Boston, MA 02111  
 United States  
 Pathobiology ( PATHOBIOLOGY ) (Switzerland) 1992, 60/1 (10-18)  
 CODEN: PATHE ISSN: 1015-2008  
 DOCUMENT TYPE: Journal; Article  
 LANGUAGE: ENGLISH SUMMARY LANGUAGE: ENGLISH

Intracellular iron can be stored in the protein shell of ferritin to protect the cell against the toxic action of the iron. In response to increased cell iron, more ferritin subunits are synthesized using translational and transcriptional mechanisms. Translational control involves a unique **stem-loop** structure in the 5' untranslated region of the subunit messengers. When iron level is low, a protein binds to this **stem-loop** structure and prevents translation. When intracellular iron level rises, the repressor protein is discharged and the large population of messengers begins to translate subunits. Similar **stem-loop** motifs occur in the 3' untranslated region of the transferrin receptor messenger where they regulate breakdown of the receptor mRNA. Finally, the presence of excess iron preferentially stimulates transcription of more ferritin message of one type (L-mRNA) which produces ferritin shells favoring iron storage. In this way, protection of the cell against iron excess is enhanced by coordinate changes in rate of synthesis of ferritin mRNA of the L-type, by release of ferritin mRNA stored in the cytoplasm, and by a reduction in the number of receptors for accepting iron into the cell. The application of these principles with reference to malignant cells is discussed.

?  
 PLEASE ENTER A COMMAND OR BE LOGGED OFF IN 5 MINUTES  
 ? ds

Set	Items	Description
S1	10500	LOOP AND ((DOUBLE (W) STRANDED) OR STEM)
S2	7314	S1 AND RNA
S3	38	S2 AND INTERLEUKIN
S4	27	RD (unique items)
S5	1243	LOOP AND (DOUBLE (W) STRANDED)
S6	685	S5 AND RNA
S7	2	S6 AND INTERLEUKIN
S8	2	RD (unique items)
S9	6	S5 AND INTERLEUKIN
S10	4	RD (unique items)

? e au=ecker

Ref	Items	Index-term
E1	1	AU=ECKENWILER L
E2	1	AU=ECKENWILER L.
E3	0	*AU=ECKER
E4	39	AU=ECKER A
E5	1	AU=ECKER A D
E6	23	AU=ECKER A.
E7	2	AU=ECKER A.D.
E8	1	AU=ECKER ACHIM
E9	3	AU=ECKER AD
E10	1	AU=ECKER ALFRED
E11	4	AU=ECKER AXEL
E12	14	AU=ECKER B

Enter P or PAGE for more

? p

Ref	Items	Index-term
E13	9	AU=ECKER B.
E14	2	AU=ECKER BERNHARD
E15	1	AU=ECKER BP
E16	1	AU=ECKER BRUCE
E17	19	AU=ECKER C
E18	3	AU=ECKER C P
E19	6	AU=ECKER C.
E20	4	AU=ECKER C.P.
E21	1	AU=ECKER CH.
E22	1	AU=ECKER CHRISTA
E23	3	AU=ECKER CHRISTIAN
E24	2	AU=ECKER CP

Enter P or PAGE for more

? p

Ref	Items	Index-term
E25	11	AU=ECKER D
E26	1	AU=ECKER D E
E27	95	AU=ECKER D J
E28	1	AU=ECKER D J J
E29	7	AU=ECKER D M
E30	8	AU=ECKER D.
E31	54	AU=ECKER D.J.
E32	8	AU=ECKER D.M.
E33	1	AU=ECKER DANIEL
E34	1	AU=ECKER DAVID
E35	34	AU=ECKER DAVID J
E36	2	AU=ECKER DAWN

Enter P or PAGE for more

? s e25 or e27 or e28or e30 or e31 or e34 or e35

	11	AU=ECKER D
	95	AU=ECKER D J
	0	E28OR E30
	54	AU=ECKER D.J.
	1	AU=ECKER DAVID
	34	AU=ECKER DAVID J
S11	195	AU="ECKER D" OR AU="ECKER D J" OR E28OR E30 OR AU="ECKER D.J." OR AU="ECKER DAVID" OR AU="ECKER DAVID J"

? rd

...examined 50 records (50)  
 ...examined 50 records (100)  
 ...examined 50 records (150)  
 ...completed examining records

· S12 140 RD (unique i )  
? 'ds

Set	Items	Description
S1	10500	LOOP AND ((DOUBLE (W) STRANDED) OR STEM)
S2	7314	S1 AND RNA
S3	38	S2 AND INTERLEUKIN
S4	27	RD (unique items)
S5	1243	LOOP AND (DOUBLE (W) STRANDED)
S6	685	S5 AND RNA
S7	2	S6 AND INTERLEUKIN
S8	2	RD (unique items)
S9	6	S5 AND INTERLEUKIN
S10	4	RD (unique items)
S11	195	AU="ECKER D" OR AU="ECKER D J" OR E28OR E30 OR AU="ECKER D- .J." OR AU="ECKER DAVID" OR AU="ECKER DAVID J"
S12	140	RD (unique items)

? s s12 and s1

140 S12  
10500 S1  
S13 4 S12 AND S1  
? rd

...completed examining records  
S14 4 RD (unique items)  
? t s14/7/1-4

14/7/1 (Item 1 from file: 5)  
DIALOG(R)File 5:Biosis Previews(R)  
(c) 2001 BIOSIS. All rts. reserv.

08768848 BIOSIS NO.: 199395058199  
Implication of RNA structure on antisense oligonucleotide hybridization kinetics.  
AUTHOR: Lima Walt F; Lonja Brett P; **Ecker David J**; Freier Susan M(a  
AUTHOR ADDRESS: (a)Dep. Molecular, Cellular Biology, Isis Pharmaceuticals,  
2280 Faraday Avenue, Carlsbad, Calif. 92  
JOURNAL: Biochemistry 31 (48):p12055-12061 1992  
ISSN: 0006-2960  
DOCUMENT TYPE: Article  
RECORD TYPE: Abstract  
LANGUAGE: English

ABSTRACT: A 47-nucleotide transcript of the activated Ha-ras gene was prepared and determined, by enzymatic structure mapping, to form a stable hairpin structure. Six antisense decaribonucleotides were designed, and association constants (K-a) for the hairpin- and length-matched complements were measured. Two of the antisense oligonucleotides targeted to the **loop** had nearly equal affinity for the transcript compared to the complement. The others, including one oligonucleotide complementary to the 3' side of the single-stranded **loop**, bound 10-5-10-6-fold less tightly to the transcript than to the short complement. We propose the difference in affinity is due to the target structure, both the secondary structure of the **stem** and the structure in the **loop**. Measurement of the bimolecular association rate constant, k-1, and the dissociation rate constant, k-1, for these oligonucleotides indicates the observed relationship between affinity and structure is primarily due to k-1.

14/7/2 (Item 2 from file: 5)  
DIALOG(R)File 5:Biosis Previews(R)  
(c) 2001 BIOSIS. All rts. reserv.

08376901 BIOSIS NO.: 0000941005

ENHANCEMENT OF RIBOSOMAL FRAMESHIFTING BY OLIGONUCLEOTIDES TARGETED TO THE  
HIV GAG-POL REGION

AUTHOR: VICKERS T A; ECKER D J

AUTHOR ADDRESS: ISIS PHARM., 2280 FARADAY AVE., CARLSBAD, CALIF. 92008.

JOURNAL: NUCLEIC ACIDS RES 20 (15). 1992. 3945-3953. 1992

FULL JOURNAL NAME: Nucleic Acids Research

CODEN: NARHA

RECORD TYPE: Abstract

LANGUAGE: ENGLISH

ABSTRACT: The pol gene of all retroviruses is expressed as a gag-pol fusion protein which is proteolytically processed to produce all viral enzymes. In the human immunodeficiency virus (HIV), the gag and pol genes overlap by 241 nucleotides with pol in the -1 phase with respect to gag. The gag-pol fusion is produced via a -1 ribosomal frameshifting event that brings the overlapping, out-of-phase gag and pol genes into translational phase. Frameshifting occurs at a so called 'shift site' 8-10 nucleotides upstream of a hairpin **loop** which may play a role in the regulation of frameshifting. We have fused this region of HIV-1 to the 5' end of the firefly luciferase reporter gene in order to quantitatively measure ribosomal frameshifting both in cells and by in vitro translation. A series of 2'-O-methyl oligonucleotides was designed to specifically bind the sequences which flank the gag-pol hairpin. Ribosomal frameshifting is enhanced up to 6 fold by those oligonucleotides which bind the area just 3 to the **stem**. Oligonucleotides which binds 5' to the **stem** have no effect on frameshift efficiency. In addition, we have constructed a series of fusion genes which mimic the effect of the bound oligonucleotides with intramolecular hairpins. The results suggest that increasing RNA secondary structure downstream of the shift site increases the frequency of ribosomal frameshifting, and that this effect can be mimicked by antisense oligonucleotides.

14/7/3 (Item 3 from file: 5)

DIALOG(R)File 5:Biosis Previews(R)

(c) 2001 BIOSIS. All rts. reserv.

07750102 BIOSIS NO.: 000092063823

INHIBITION OF HIV-LTR GENE EXPRESSION BY OLIGONUCLEOTIDES TARGETED TO THE  
TAR ELEMENT

AUTHOR: VICKERS T; BAKER B F; COOK P D; ZOUNES M; BUCKHEIT R W JR; GERMANY  
J; ECKER D J

AUTHOR ADDRESS: ISIS PHARM., 2280 FARADAY AVE., CARLSBAD, CALIF. 92008.

JOURNAL: NUCLEIC ACIDS RES 19 (12). 1991. 3359-3368. 1991

FULL JOURNAL NAME: Nucleic Acids Research

CODEN: NARHA

RECORD TYPE: Abstract

LANGUAGE: ENGLISH

ABSTRACT: All human immunodeficiency virus mRNAs contain a sequence known as TAR (trans-activating responsive sequence). The TAR element forms a stable RNA **stem-loop** structure which binds the HIV tat (trans-activator) protein and mediates increased viral gene expression. In principle, molecules which bind to the TAR RNA structure would inhibit trans-activation by perturbing the native RNA secondary structure. We have constructed a series of phosphodiester and phosphorothioate antisense oligonucleotides which specifically bind to the HIV TAR element. Specific binding to the TAR element was demonstrated in vitro with enzymatically synthesized TAR RNA. The TAR-directed phosphorothioates inhibited trans-activation in a sequence-dependent fashion in a cell culture model using an HIV LTR/human placental alkaline phosphatase gene fusion and tat protein supplied in trans. The molecules also inhibited HIV replication in both acute and chronically infected viral assays, but without sequence specificity. We have constructed a series of vectors consisting of the MMTV promoter and 5'-untranslated region of four different mRNAs, including the TAR region, to study the

"effect of TAR on gene expression in heterologous systems. The results suggest that, in the absence of the HIV LTR, the TAR element has a repressive effect on gene expression, which is relieved by tat.

14/7/4 (Item 1 from file: 357)

DIALOG(R)File 357:Derwent Biotechnology Abs

(c) 2001 Derwent Publ Ltd. All rts. reserv.

0236099 DBA Accession No.: 99-06200 PATENT

New oligonucleotides for modulating gene expression by RNA mimicry - RNA probe for use in disease diagnosis and RNA oligonucleotide for use in HIV virus and retro virus disease therapy

AUTHOR: **Ecker D J**; Bruice T W; Vickers T

CORPORATE SOURCE: Carlsbad, CA, USA.

PATENT ASSIGNEE: Isis-Pharm. 1999

PATENT NUMBER: US 5874564 PATENT DATE: 990223 WPI ACCESSION NO.: 99-180068 (9915)

PRIORITY APPLIC. NO.: US 461418 APPLIC. DATE: 950605

NATIONAL APPLIC. NO.: US 461418 APPLIC. DATE: 950605

LANGUAGE: English

ABSTRACT: Oligonucleotides (or their analogs) able to mimic the secondary or tertiary structure of RNA molecules, especially mRNA, are claimed. Specific oligonucleotides which mimic the HIV virus trans-acting responsive element (TAR) are claimed. Also claimed are oligonucleotides of 8-50 residues containing the 4 disclosed RNA sequences with virucide activity. The RNA mimicry oligonucleotides mimic particular strands of RNA, especially mRNA, containing secondary structures important for RNA/protein interactions. The interaction of proteins with mimic molecules minimizes the interactions of proteins with regulatory RNA. A 2'-O-methyl oligonucleotide analog 29-mer forms a truncated HIV virus TAR **stem/loop** structure that binds to the tat peptide in vitro. This oligonucleotide showed high inhibition of HIV virus gene expression. The oligonucleotides are useful for gene expression modification and in gene therapy of virus and retro virus infections e.g. AIDS. The oligonucleotides can also be used as RNA probes in diagnosis and research. (21pp)

? logoff

24oct01 13:56:32 User233835 Session D513.3

\$2.59 0.808 DialUnits File155

\$0.00 16 Type(s) in Format 6

\$1.80 9 Type(s) in Format 7

\$1.80 25 Types

\$4.39 Estimated cost File155

\$5.03 0.899 DialUnits File5

\$0.00 5 Type(s) in Format 6

\$4.95 3 Type(s) in Format 7

\$4.95 8 Types

\$9.98 Estimated cost File5

\$3.51 0.278 DialUnits File357

\$0.00 2 Type(s) in Format 6

\$7.05 3 Type(s) in Format 7

\$7.05 5 Types

\$10.56 Estimated cost File357

\$8.58 1.010 DialUnits File73

\$0.00 10 Type(s) in Format 6

\$14.10 6 Type(s) in Format 7

\$14.10 16 Types

\$22.68 Estimated cost File73

OneSearch, 4 files, 2.994 DialUnits FileOS

\$1.15 TYMNET

\$48.76 Estimated cost this search

\$49.32 Estimated total session cost 3.202 DialUnits

Logoff: level 01.10.01 D 13:56:32